



**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460**

**OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES**

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MEMORANDUM

SUBJECT: Tolerance Review of Compounds of the Citric Acid Cycle (Kreb's Cycle) as Inert Ingredients in Terrestrial and/or Aquatic Agricultural and Non-Agricultural Uses

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This memorandum transmits the Environmental Fate and Effects Division's (EFED) exposure and risk assessment of compounds of the Citric Acid Cycle (also known as the Kreb's Cycle). The assessment is based on readily available information from the Agency and peer reviewed public literature sources. Information contained in these sources permits EFED to conduct a qualitative assessment of environmental exposures and risks.

If you should have any questions concerning the information within, please contact me at 305-7346.

Conclusions

A review of the readily available information on the compounds that make up the Citric Acid Cycle (also known as the Krebs's Cycle) is sufficient to conduct a qualitative assessment of the potential exposures and risks associated with their use as pesticide inert ingredients. Environmental loadings are attributable to natural (plants and animal materials) and anthropogenic (food additives, drugs, and related products) sources. Available data indicate that they rapidly dissociate in the aquatic environment at environmentally relevant pH's to the corresponding acid (anion) and its respective cation (H^+ , Ca^+ , K^+ , Na^+ , NH_4^+ , Mg^{2+} , among others). Anions of the respective compounds undergo aerobically mediated mineralization in days to weeks; mineralization is complete degradation to CO_2 and water. Half-lives are anticipated to be shorter. Under anaerobic conditions, the anions are expected to mineralize in a matter of weeks. These compounds are expected to be predominantly found in the non-absorbed/adsorbed state in aquatic environments based on fugacity modeling.

Photodegradation will also play a role in the dissipation of the compounds, but, generally will occur at a lower rate, i.e., half-lives may be longer than biodegradation. Mobility of the anions is expected to be high based on adsorption estimates, however, migration to ground water should be substantially mediated through their rapid biodegradation, volatilization, or through their uptake and utilization within plant cells. Runoff to surface is expected to dominate the non-degradation pathways of dissipation. Partitioning to air is expected to be low from water sources (based on the Henry's Law constant) and high from dryer surfaces such as soils (based on volatility). Bioconcentration is not expected to occur.

Shallow aquifer ground water concentrations of the anions of the citric acid cycle may reach low part per milligram (ppm) under conditions of exaggerated surface contamination (1-100 ppm) such as those observed beneath landfills and wood-treatment facilities. However, they are generally found in the low parts per billion (ppb) elsewhere (<100 ppb) some of which is attributable to natural sources (<http://toxnet.nlm.nih.gov>). Surface water concentrations (fresh water and marine-estuarine) have been reported to reach the low- to mid-ppb range (10-300 ppb). As with ground water occurrence, surface water concentrations can be attributed to natural sources as well. No data were available to determine the effects of drinking water treatment on concentrations of the anions at the consumer tap. Based on the strong oxidizing nature of the chlorination step of treatment utilities and the chemical structure of these compounds, it is unlikely that these compounds will be found in treated water at concentrations equivalent to those found in the environment. There are no ambient water quality criteria or drinking water maximum contaminant or health advisory levels for these compounds.

Based on the Agency's toxicity categories, as a group, these compounds would be classified as slightly toxic to practically non-toxic to aquatic organisms. Terrestrial organism toxicity, using mammal data as a surrogate for the absence of avian data, indicate that these compounds would be classified as slightly toxic to practically non-toxic except via the inhalation route where toxicity is significantly greater, one or more orders of magnitude greater depending

on the compound. At anticipated environmental concentrations, substances associated with the citric acid cycle are not expected to exceed the available toxicity data for both aquatic and terrestrial organisms to include exposures and risks via the inhalation route.

Introduction

The compounds contained in this review are grouped together because of their close structural relationships, carboxylic acids and their salts, and the role each plays in cell metabolism, specifically in the tricarboxylic acid cycle (also known as the citric acid or Krebs's cycle). The compounds subject to this review are as follows: acetic acid, acetic acid ammonium salt, acetic acid calcium salt, acetic acid magnesium salt, acetic acid manganese salt, acetic acid potassium salt, acetic acid sodium salt, citric acid, citrate, citric acid sodium salt, citric acid sodium salts, fumaric acid and malic acid. These compounds are naturally occurring in foods and essential to normal metabolic processes.

Citric, fumaric and malic acid play key roles in the metabolic energy system called the Citric Acid Cycle or Krebs's Cycle. The cycle consists of a series of enzymatic chemical reactions occurring within the cell that are responsible for the final breakdown of food molecules to form carbon dioxide, water, and energy. Citric, fumaric, and malic acid are the integral components in this reaction, each being used over and over again to produce energy. They are not found in appreciable amount as waste products from animals as they play a key role in the acid-base balance within animal systems. This process is active in all animals and higher plants and is carried out in the mitochondria (American Chemical Council, 2001).

Acetic Acid and its Salts

The chemical structures, physical-chemical properties, environmental fate behavior, and aquatic and terrestrial toxicity of these compounds are similar. Acetic acid and its salts undergo dissociation in aqueous media at pH's commonly found in the environment to the acetate anion and the respective cations. The toxicity of each compound is driven by acetate, with the cations playing a minor role. Table 1 provides key environmental physical-chemical properties and fate data. Biodegradation appears to be the most significant removal mechanism following the dissociation of the compound into the acetate anion and respective salt. Data indicate that acetic acid and sodium acetate (acetic acid, sodium salt) photodegrade, although the rate is substantially slower than that of biodegradation. Fugacity modeling predicts 73% of any acetic acid released to the environment would partition to water based on solubility and partition coefficients, with the remainder partitioning into the air. Therefore, the data suggest that acetic acid and its salts are not persistent in the environment.

Ecotoxicity data for aquatic and terrestrial animals are available for four of the seven compounds, Table 1. The ecotoxicity data indicate that these compounds are slightly to practically nontoxic on an acute basis. The three remaining salts are closely related to the other salts in structure, properties and behavior and would be expected to have similar toxicity. Terrestrial

animal toxicity based on available mouse and rat data would indicate acetic acid is practically non-toxic on an acute basis and no chronic effects were observed in available studies.

Citric Acid and its Salts

Citric acid and its salts are comprised of four compounds, which include citric acid, sodium citrate, tripotassium citrate, and trisodium citrate. The chemical structures and available data indicate that the physical-chemical properties, environmental fate behavior, and aquatic and terrestrial toxicity of these four compounds are similar, Table 2. As in the case of the other acids and salts in this category, citric acid and its salts undergo dissociation in aqueous media into the citrate anion and the respective cations. The toxicity of each compound is driven by citrate, with the cations playing a minor role.

These compounds are highly water soluble and of moderate to low volatility. Data on the environmental fate of citric acid and its trisodium salt indicate that citric acid and its salts dissociate into their respective cations and the citrate anion, which is subsequently readily biodegraded. Studies indicate that citric acid and its trisodium salt are readily biodegraded (90-98% degradation after 48 hours). Fugacity modeling predicts that 100% of any citric acid released to the environment would partition to water based on solubility and partitioning coefficients. Therefore, the existing data indicates that citric acid and its salts are not persistent in the environment.

Aquatic toxicity data for fish, *Daphnia* and algae are available for citric acid and its trisodium salt and indicate that these compounds are practically non-toxic on an acute basis, with LC₅₀ values ranging from 120 to >18,000 mg/L, Table 2. Terrestrial animal toxicity based on available mouse data would indicate citric acid is practically non-toxic on an acute basis and no chronic effects were observed in available studies.

Fumaric Acid

Available data indicate that fumaric acid is highly soluble in water and has low volatility. Fugacity modeling predicts that virtually all (99.8%) of any fumaric acid released to the environment would partition to water based on solubility and partitioning coefficients. Fumaric acid dissociates into fumarate and hydrogen ion followed by fumarate undergoing degradation by both biotic and abiotic mechanisms. Nearly complete biodegradation was observed after 21 days under aerobic conditions, Table 3. These data indicate that fumaric acid is not persistent in the environment.

Aquatic LC₅₀ values for fish and *Daphnia* were greater than 200 mg/L. The value for the more sensitive algae was 41 mg/L. These data indicate that fumaric acid is slightly to practically non-toxic to aquatic animals and plants on an acute basis. Terrestrial animal toxicity based on

available rat data would indicate fumaric acid is practically non-toxic on an acute basis and no chronic effects were observed in available studies.

Malic Acid

Malic acid is highly soluble in water and has a low volatility. Malic acid dissociates into malate and hydrogen ion and pH's commonly found in the aquatic environment. Malate is considered readily biodegradable in soil and water and fugacity modeling predicts that 100% of any malic acid released to the environment would partition to water based on solubility and partitioning coefficients, Table 3. Based on these data, malic acid is not likely to be persistent in the environment.

Data on the aquatic toxicity of malic acid to aquatic invertebrates are available. No data on toxicity to fish and algae were available. A 48-hour LC₅₀ for *Daphnia magna* was 240 mg/L, which classifies malic acid as practically non-toxic to aquatic invertebrates on an acute basis. Given this data and the aquatic toxicity data for the structurally related compounds in this category, malic acid is likely to be practically non-toxic to other aquatic species on an acute basis. Terrestrial animal toxicity based on available rat data would indicate malic acid is practically non-toxic on an acute basis and no chronic effects were observed in available studies.

References

American Chemistry Council, 2001. U.S. High Production Volume (HPV) Chemical Challenge Program, Assessment Plan for Acetic Acid and Salts Category. Prepared by: American Chemistry Council, Acetic Acid and Salts Panel, June 28, 201.

TOXNET 2002. Online Scientific Search Engine, National Library of Medicine, National Institutes of Health. Search results for Acetic Acid and salts, Citric Acid and salts, and Fumaric Acid.

U.S. EPA, 1992. Reregistration Eligibility Decision (RED): Citric Acid Fact Sheet. Office of Prevention, Pesticides and Toxic Substances. EPA-738-F-92-017. June 1992.

Table 1. Chemical Properties of Acetic Acid and Salts

Chemical Property	Acetic Acid	Acetic Acid, Ammonium salt	Acetic Acid, Calcium salt	Acetic Acid, Magnesium salt	Acetic Acid, Manganese salt	Acetic Acid, Potassium salt	Acetic Acid, Sodium salt
Vapor Pressure (mmHg)	11.4 @ 20°C	1.4×10^{-4} @ 25°C	14.7 @ 25°C	NA	NA	7.08×10^{-7} @ 25°C	7.08×10^{-7} @ 25°C
Log Kow	-0.17	-2.79	-0.97	NA	NA	-3.72	-3.72
Kd's (Koc)	0.65 (228) Clastic mud 0.085 (6.5) muddy sand 0.046 (27) carbonate sand	NA	NA	NA	NA	NA	NA
Water Solubility (g/L)	50 @ 20°C	1,480 @ 4°C	430 @ 25°C	very solu ble	soluble	2530g/L @ 25°C	365g/L @ 20°C
pKa	4.76 @ 25°C	NA	NA	NA	NA	NA	NA
Photodegradation	50% after 21 days	NA	NA	NA	NA	NA	6.6% after 17h
Biodegradation	99% after 7 days using AS	days to weeks (SAR)	Readily biodegrades	NA	NA	NA	100% after 5 days using AS
Fish Acute Toxicity (96h- LC ₅₀)	75mg/L (Lowest value - Bluegill sunfish)	238mg/L (mosquito fish)	NA	NA	NA	>6100mg/L (rainbow trout)	100mg/L (zebra fish)
<i>Daphnia</i> Acute Toxicity	65mg/L (48h- EC ₅₀)	NA	NA	NA	NA	7170mg/L (24h- LC ₅₀)	>1000mg/L (48h- EC ₅₀)

Algae Toxicity	4000mg/L (8-day growth inhibition)	NA	NA	NA	NA	NA	2460mg/L after 60-h growth inhibition)
Mammal Acute Oral (LD ₅₀)	4960 mg/kg-bw (mouse)	NA	4280mg/kg-bw (rat)	8610mg/kg-bw (rat)	3730mg/kg-bw (rat)	3250mg/kg-bw (rat)	3530mg/kg-bw (rat)

AS: Activated Sludge. SAR: Structure Activity Relationship. NA: Not Available.

Table 2. Chemical Properties of Citric Acid and Salts

Chemical Property	Citric Acid	Citric Acid, Sodium salt	Citric Acid, Tripotassium salt	Citric Acid, Trisodium salt
Vapor Pressure (mmHg@25°C)	3.7x10 ⁻⁹ @25°C	NA	NA	2.09x10 ⁻¹² @25°C
Log Kow	-1.72	NA	NA	-0.28
Water Solubility (g/L @25°C)	1330g/l @20°C	NA	NA	~425g/L @25°C
pKa	pK1: 3.13; pK2: 4.76; pK3: 6.4	NA	NA	NA
Photodegradation	NA	NA	NA	NA
Biodegradation	98% after 48-h using domestic sewage	NA	NA	90% after 48-h using AS
Fish Acute Toxicity (96h-LC ₅₀)	1516mg/L (Bluegill sunfish)	NA	NA	>18000-32000mg/L (guppy)
<i>Daphnia</i> Acute Toxicity	120mg/L (72h-EC ₅₀)	NA	NA	5600-10000mg/L (48h-EC ₅₀)
Algae Toxicity	640mg/L 8-day growth inhibition)	NA	NA	>18000-32000mg/L (96h-EC ₅₀)
Mammal Acute Oral	5790mg/kg-bw (mouse)	7100mg/kg-bw (mouse)	NA	NA

Table 3. Chemical Properties of Fumaric and Malic Acid

Chemical Property	Fumaric Acid	Malic Acid
Vapor Pressure (mmHg@25°C)	1.54×10^{-4}	4.6×10^{-6}
Log Kow	0.33 @23°C	-1.26
Water Solubility (g/L @25°C)	7g/L	592g/L
pKa	pK1: 3.02; pK2:4.46 @18°C	pK1: 3.4; pK2: 5.05
Photodegradation	50% degradation after 7.3h	50% degradation after 2 days
Biodegradation	98% after 21 days using domestic sewage	readily biodegrades
Fish Acute Toxicity	245mg/L (48h-LC ₅₀ - zebra fish))	NA
<i>Daphnia</i> Acute Toxicity	212 mg/L (48h-EC ₅₀)	240mg/L (48h-EC ₅₀)
Algae Toxicity	41mg/L (72h-EC ₅₀ - green algae)	NA
Rat Acute Oral	9300mg/kg-bw (female rat)	1600-3200mg/kg-bw (mouse, rat)